

9-19-96

MRID No. 439784-01

DATA EVALUATION RECORD  
S 72-2 - ACUTE EC<sub>50</sub> TEST WITH A FRESHWATER INVERTEBRATE

1. CHEMICAL: Ethoxyquin PC Code No.: 055501

2. TEST MATERIAL: Ethoxyquin technical Purity: 99.1%

3. CITATION:

Authors: K.R. Drottar and J.P. Swigert  
Title: Ethoxyquin: A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*).  
Study Completion Date: April 4, 1996

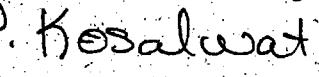
Laboratory: Wildlife International Ltd., Easton, MD  
Sponsor: Oregon, Washington, and California Pear Bureau, Portland, OR  
Laboratory Report ID: 442A-102

MRID No.: 439784-01  
DP Barcode: D225526

4. REVIEWED BY: Mark Mossler, M.S., Toxicologist,  
KBN Engineering and Applied Sciences, Inc.,

Signature:  Date: 7/5/96

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,  
KBN Engineering and Applied Sciences, Inc.,

Signature:  Date: 7/5/96

5. APPROVED BY:  Date: 9/19/96

Signature:

6. STUDY PARAMETERS:

Age of Test Organism: <24 hours

Definitive Test Duration: 48 hours

Study Method: Flow-through

Type of Concentrations: Mean measured

7. CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an acute toxicity study using freshwater invertebrates. The EC<sub>50</sub> value of 2.0 ppm ai classifies ethoxyquin technical as moderately toxic to *Daphnia magna*.

Results Synopsis

EC<sub>50</sub>: 2.0 ppm ai

NOEC: 1.0 ppm ai

95% C.I.: 1.7 - 2.9 ppm ai  
Probit Slope: N/A

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**8. ADEQUACY OF THE STUDY:**

- A. Classification: Core
- B. Rationale: N/A
- C. Repairability: N/A

**9. GUIDELINE DEVIATIONS:**

1. The pH of the dilution water (8.2-8.3) was greater than recommended (7.2-7.6).
2. The hardness of the dilution water (140 mg/L as CaCO<sub>3</sub>) was greater than recommended (40-48 mg/L as CaCO<sub>3</sub>).

**10. SUBMISSION PURPOSE:****11. MATERIALS AND METHODS:****A. Test Organisms**

Guideline Criteria	Reported Information
<b>Species</b> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
All organisms are approximately the same size and weight?	Not reported
<b>Life Stage</b> Daphnids: 1 <sup>st</sup> instar (<24 h). Amphipods, stoneflies, and mayflies: 2 <sup>nd</sup> instar. Midges: 2 <sup>nd</sup> & 3 <sup>rd</sup> instar.	1 <sup>st</sup> instar (<24 h)
<b>Supplier</b>	In-house cultures
All organisms from the same source?	Yes

**B. Source/Acculturation**

Guideline Criteria	Reported Information
<b>Acclimation Period</b> Minimum 7 days	Cultures maintained under conditions similar to test

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Guideline Criteria	Reported Information
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Feeding</u> No feeding during the study.	No feeding during the study
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing.	Not reported

**C. Test System**

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water.	Moderately-hard well water, filtered and aerated
<u>Does water support test animals without observable signs of stress?</u>	Yes
<u>Water Temperature</u> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	19.9-20.4°C
<u>pH</u> Prefer 7.2 to 7.6.	8.2-8.3
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 <sup>st</sup> 48 h and ≥ 40% during 2 <sup>nd</sup> 48 h, flow-through: ≥ 60%.	90-101% of saturation during the test
<u>Total Hardness</u> Prefer 40 to 200 mg/L as CaCO <sub>3</sub> .	140 mg/L as CaCO <sub>3</sub>

Guideline Criteria	Reported Information
<b>Test Aquaria</b> 1. <u>Material:</u> Glass or stainless steel. 2. <u>Size:</u> 250 mL (daphnids and midges) or 3.9 L (1 gal). 3. <u>Fill volume:</u> 200 mL (daphnids and midges) or 2-3 L.	Glass 300 mL 300 mL
<b>Type of Dilution System</b> Must provide reproducible supply of toxicant.	Continuous-flow diluter
<b>Flow Rate</b> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	14 volume replacements every 24 hours
<b>Biomass Loading Rate</b> Static: $\leq 0.8$ g/L at $\leq 17^{\circ}\text{C}$ , $\leq 0.5$ g/L at $> 17^{\circ}\text{C}$ ; flow-through: $\leq 1$ g/L/day.	N/A
<b>Photoperiod</b> 16 hours light, 8 hours dark.	16 hours light, 8 hours dark
<b>Solvents</b> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests.	Solvent: methanol Maximum conc.: 0.1 mL/L

**D. Test Design**

Guideline Criteria	Reported Information
<b>Range Finding Test</b> If $\text{EC}_{50} > 100$ mg/L, then no definitive test is required.	Yes, but results were not reported
<b>Nominal Concentrations of Definitive Test</b> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	Control, solvent control, 0.39, 0.65, 1.1, 1.8, and 3.0 mg ai/L

<b>Number of Test Organisms</b> Minimum 20/level, may be divided among containers.	20 per level, 10 per replicate
<b>Test organisms randomly or impartially assigned to test vessels?</b>	Yes
<b>Water Parameter Measurements</b> 1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C. 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	Temperature measured continuously in one negative control chamber and in each chamber at initiation and termination DO and pH measured daily in alternating replicates
<b>Chemical Analysis</b> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Yes

**12. REPORTED RESULTS:****A. General Results**

Guideline Criteria	Reported Information
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Yes
<b>Control Mortality</b> Static: ≤10% Flow-through: ≤5%	0% mortality in the dilution water and solvent controls
<b>Percent Recovery of Chemical</b>	90-97%
<b>Raw data included?</b>	Yes

**Mortality**

Nominal	Mean Measured	Number of Organisms	Cumulative Number Dead	
			24	48
Control	<0.02	20	0	0
Solvent Control	<0.02	20	0	0
0.39	0.35	20	0	0
0.65	0.60	20	0	0
1.1	1.0	20	0	0
1.8	1.7	20	0	5
3.0	2.9	20	6	20

Other Significant Results: Some of the surviving daphnids at the 1.8 ppm nominal treatment level were noted to be lethargic.

**B. Statistical Results**

Method: Binomial method

48-hr EC<sub>50</sub>: 2.0 ppm ai  
Probit Slope: N/A

95% C.I.: 1.7 - 2.9 ppm ai  
NOEC: 1.0 ppm ai

**13. VERIFICATION OF STATISTICAL RESULTS:**

Parameter	Result
Binomial Test EC <sub>50</sub> (C.I.)	2.0 (1.7 - 2.9) ppm ai
Moving Average Angle EC <sub>50</sub> (95% C.I.)	N/A
Probit EC <sub>50</sub> (95% C.I.)	N/A
Probit Slope	N/A
NOEC	1.0 ppm ai

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14. **REVIEWER'S COMMENTS:** This study is scientifically sound, fulfills the guideline requirements for an acute toxicity study using freshwater invertebrates, and can be classified as Core. The EC<sub>50</sub> value of 2.0 ppm ai classifies ethoxyquin technical as moderately toxic to *Daphnia magna*.

mossler ethoxyquin daphnia magna 7-2-96.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2.9	20	20	100	9.536742E-05
1.7	20	5	25	2.069473
1	20	0	0	9.536742E-05
.6	20	0	0	9.536742E-05
.35	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.7 AND 2.9 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.962665

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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